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博士学位论文

温度与光周期调控下南北极甲藻种类 *Polarella glacialis* Montresor et al. 1998 的生长特征

Growth of *Polarella glacialis*, a bipolar dinoflagellate, at increased temperatures and varied light-dark cycles

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摘 要

近年来全球气候变化趋势越加严峻，特别在南北极地区。本文以 *Polarella glacialis*——一种对高纬度南极海冰及北冰洋水层甲藻产量有重要贡献而其生理特性尚属空白的两极甲藻为代表，通过观察不同温度和光周期下 *P. glacialis* 的生长、细胞周期特征与 Rubisco 和类 PCNA 蛋白等的表达量变化以阐明该藻在极地极端光周期下的生长特性，回答以该藻为代表的极地藻类能否通过改变或调整表型特性来适应逐渐升高的环境温度。

对已成功分离培养的 *P. glacialis* 的两藻株 CCMP2088、CCMP1383 设置了不同的温度和光照周期：4°C 和 14L/10D 光照周期、4°C 和 10L/14D 光照周期、10°C 和 14L/10D 光照周期、15°C 和 14L/10D 光照周期、20°C 和 12L/12D 光照周期、4°C 和 24L 连续光照、4°C 和 24D 连续黑暗，应用显微镜观察藻细胞数量的变化、流式细胞仪分析其细胞周期以及 Western 免疫印迹分析蛋白的表达量变化，主要结果如下：

1、藻株 CCMP2088 与 CCMP1383 在显微镜下形态差别不大，细胞密度达到约 10^5cells•mL^{-1} 或培养温度升高时许多细胞聚集成团，并由粘性聚合物包被附于瓶壁或瓶底，并可见孢子细胞。

2、推荐培养温度 4°C 与 14L/10D 光周期下，CCMP2088 藻株起始密度为 5.1×10^4cells•mL^{-1}，先经历 4 天的滞后期后进入指数生长期，22 天内的平均生长率为 0.10 ± 0.17d^{-1}，减速增长期或称线性生长期持续超过 23 天，平均生长率 0.027 ± 0.043d^{-1}，两个时期并不明显区分。减速生长期内藻株的细胞周期长度大于 24hrs。专抗 *Prorocentrum minimum* Rubisco 蛋白的抗体检测到~53kDa 的条带，而专抗 *Pfiesteria piscicida* PCNA 蛋白的抗体检测到～55kDa 的条带，远大于已报道的大多数甲藻的 PCNA 蛋白（33～36kDa），需要进一步的实验以确定该蛋白成分，本文将其暂定为类 PCNA 蛋白。指数生长期内 S 与 G_2 期占的细胞数百分比以及

Rubisco 与类 PCNA 单位蛋白的表达量均高于减速生长期，显示藻细胞在指数生长期内的生长生理活性高于减速生长期。减速生长期内始终能检测到两种蛋白，并有昼夜节律，Rubisco 和 PCNA 蛋白表达量的峰值分别位于光照期前 4hrs 和光照期开始后 4hrs。

CCMP2088 藻株在 4°C 与 10L/14D 光周期下培养至指数生长期时，G_2 期位于光照期开始后的 1.5-5.5hrs，类 PCNA 蛋白的单位表达量在 G_2 期初达到峰值，而 Rubisco 的单位蛋白表达量峰值出现于 6hrs 后（暗阶段）。CCMP1383 藻株在 4°C 与 10L/14D 光周期下培养至指数生长期的平均生长率为 $0.082\pm0.24d^{-1}$，略低于相同培养条件下的 CCMP2088 藻株（$0.10\pm0.11d^{-1}$）。接近平台期时 G_2%恒定于较低水平（平均值 0.67±0.59%），两种关键蛋白的表达具有昼夜节律，绝对表达量峰值均出现于进入光阶段的 3hrs 时，而最低值均位于 6hrs 前（暗阶段）。

3、在光周期同样是 14L/10D 的条件下，当培养温度升高至 10°C、15°C 时，藻株 CCMP2088 经历了 4 天的滞后期后细胞数以 $-0.2d^{-1}$ 左右的速度持续减少，13-15 天后细胞数量趋于稳定。10°C 下培养的藻株 S%、G_2%逐渐增加最终高于 4°C 培养的藻株，而在 15°C 培养的藻株 S%逐渐增加而 G_2%大幅降低并趋于 0。两种蛋白的表达量先减少后当细胞数量趋于稳定时发生改变并有稳定增加的趋势，Rubisco 抗体检测到~14.8kDa 的条带，可能是 Rubisco 在较高温度下的降解片断或变异，PCNA 抗体则检测到~115.5kDa 和~55kDa 两条条带，前者可能是~55kDa 蛋白的二聚体。在细胞数量稳定期，10°C、15°C 下的藻株仍有细胞周期节律和关键蛋白的节律表达，说明仍有部分藻细胞能耐受升高的培养温度，其中 15°C 对藻株生长的抑制作用较为显著。

而将两藻株置于 15°C 与 14L/10D、20°C 与 12L/12D 下培养初期(3-5d)，藻细胞大量死亡，细胞数量迅速下降，细胞的生理活动受到较大影响，G_2%保持在较低水平，甚至趋近于 0，而 S%仍存在一定的昼夜节律，两种蛋白的表达量也迅速减少，显示藻株处于温度骤升的应激状态中。培养的 11 天内细胞数量持续减少，而

藻株在 15°C 与 14L/10D 下细胞数量的减少速度更快，可能与暗阶段的持续时间增加有关。

综上所述，将 *P. glacilis* 置于较高温度(10°C、15°C、20°C)下培养，培养初期藻细胞数量下降较快，生理活动波动剧烈，G_2%水平低，蛋白表达量减少，但经过一段适应期后，部分藻细胞可能耐受升高的温度使藻细胞数量趋于稳定。由于气候变暖是一个长期渐变的过程，以 *P. glacilis* 为代表的极地藻类很有可能适应新的环境温度继续为极地甲藻产量贡献重要份额。

4、连续光照培养条件下藻细胞数量的增长速率与常规光照周期下培养的藻株相当，一昼夜内出现均匀交替的 4 个 G_2%峰和 3 个 S%峰，间隔 6－8 小时，且 S%、G_2%水平高于正常光照节律下生活的藻株，两种蛋白的表达仍有昼夜节律，峰值出现于同一个时间点，也是第一个 S%峰出现的时间，显示连续光照改变了藻细胞的正常生理节律，但藻株仍能正常生长。

连续无光条件下藻细胞数量持续减少，一昼夜内也出现了 4 个 G_2%和 S%峰 ，但峰的幅度小于连续光照培养下的藻株，总体的 G_2%水平低于其他光照条件下培养的藻株（P<0.01），两种蛋白的表达随着无光期的持续逐渐减少，显示连续黑暗不仅干扰了藻细胞正常的生理节律，也不利于藻株的生长。推测每年 *P. glacialis* 在高纬度的春季爆发（常规的光暗周期），夏季（极昼）继续生长繁荣，秋季（常规的光周期）逐渐衰退并形成大量孢子以越冬（极夜）。

关键词：*Polarella glacialis*；生长；细胞周期；Rubisco；PCNA

Abstract

Polarella glacialis, an important dinoflagellate contributes substantially to the phytoflagellate biomass and primary production in the Antarctic sea ice and the Arctic Ocean. Few physiological characteristics of it have ever been studied. In this paper we are trying to understand how *P. glacialis* acclimates the polar days and polar nights, and if it can acclimate or adjust through phenotypic responses to the increasing temperature as the climate change is getting wilder worldly, especially in the polar region, by observing its growth, cell cycle patterns and the expression of some indicative proteins like Rubisco and PCNA-like proteins at different temperatures and light/dark cycles.

The successfully cultured two strains of *P. glacialis* so far, CCMP2088 and CCMP1383 were cultured and observed at 4°C and 14L/10D cycle, 4°C and 10L/14D cycle, 10°C and 14L/10D cycle, 15°C and 14L/10D cycle, 20°C and 12L/12D cycle, 4°C and 24h L and 4°C and 24h D. And the cell cycle patterns and the expression of the indicative proteins were monitored by flow cytometry and western blotting, respectively. Here we list our main results as the following.

1. The appearance of the two strains did not show much difference under the microscope. When the culture arrived to a certain density (about 10^5cells•mL^{-1}) or was under increased temperatures, cells tended to agglomerate and adhered to the bottom surface of the culture containers. In the mature culture of *P. glacialis*, cyst-like cells were found under microscope sticking with vegetative cells covered in a certain adhesive excretions.

2. When growing at the recommended temperature of 4°C and 14L/10D cycle and starting from 5.1×10^4cells•mL^{-1}, the culture of CCMP2088 firstly experienced 4-day lag phase, then entered the logarithmic growth at a pace of 0.10 ± 0.17 d^{-1} within 22 days， followed by the decelerated growth (linear growth) of more than 23 days. The two growth phases were not clearly pronounced. A single cell cycle lasted more than 24 hours in the decelerated growth stage. Bands of ~53kDa were identified with the Ppi-Rubi

antibody developed specifically against Rubisco in *Prorocentrum minimu* and bands of ~55kDa were identified with Ppi-PCNA antibody developed specifically against PCNA in *Pfiesteria piscicida*, which were much bigger than most reported dinoflagellate PCNA (33~36kDa). Thus, it was called PCNA-like protein in this paper. Expression per unit of Rubisco and PCNA-like proteins in the exponential stage was more than in the decelerated growth stage, and the cell percentages of S and G_2 phases were higher in the logarithmic growth than in the decelerated growth, indicating the culture grew and functioned more actively in the logarthmic growth. The two proteins were found to express throughout a diel cycle and showed a circadian cycle. The expression peaks of Rubisco and PCNA-like protein were found at 4hrs before light was off and 4hrs after light was on correspondingly.

Growing at 4°C and 10L/14D cycle in the exponential stage, the G_2 phase of the culture of CCMP2088 lasted from 1.5hrs to 5.5hrs after light was on. And the amount of PCNA-like protein and Rubisco per unit arrived their peaks at the beginning of the G_2 phase and 6hrs later (in the dark period) respectively. When growing at 4°C and 10L/14D cycle, the culture of CCMP1383 grew at an average of $0.082\pm0.24d^{-1}$ in the exponential stage. G_2% was low through the diel cycle when the culture was close to the stationery stage with an average of 0.67±0.59%. Rubisco and the PCNA-like protein expressed throughout the diel cycle and showed a circadian pattern. Their peak values appeared at 3hrs after light was on while their lowest values were at 6hrs before (in the dark period).

3. When growing at 10°C and 15°C with 14L/10D cycle, CCMP2088 firstly went through a 4-day lag phase. Then the cells died at -0.2 d^{-1}. After 13-15 days, cell concentration tended to be stable. S% and G_2% of the culture growing at 10°C increased gradually and even exceeded those of the culture growing at 4°C. The 15°C culture had an increasing S% and a decreasing G_2%. The G_2% was getting close to 0. The two indicator proteins expressed less first, but went back when the cell concentration tended to be stable. Bands of ~14.8kDa were identified with Rubisco antibody and bands of ~115.5kDa and ~55kDa were identified with PCNA antibody. The bands of ~115.5kDa might be dimers of the bands of ~55kDa. At the period with stable cell densities, the cell cycle and the expression of the indicator proteins in the cultures growing at 10 and 15°C

showed circumdian pattern, indicating there were some cells can endure the enhanced temperature. However, 15°C showed greater inhibiting effect to growth of the culture.

At the beginning of culturing the two stratins at 15°C with 14L/10D and 20°C with 12L/10D (3-5d), the cell concentrations dropped briefly, cells' physiological activities were suppressed, G_2% stayed at a low level, even near to 0, and the expresson of two proteins reduced rapidly. But S% still had circadian pattern. The cultures were in temperature shock. The cell densities went down continuously in 11 days. However, the reducing rate was more at 15°C with 14L/10D, which might owe to the extended dark period in the light/dark cycle.

To conclude, when cultured at enhanced temperatures (10°C、15°C、20°C) the cell densities declined rapidly, the physiological activities were suppressed, G_2% decreased to a low level and the indicator proteins expressed less. After a certain time for the cultures to adapting, some cells might endure the temperatures and the cell densities were tended to be steady. Since the suggested global warming is a long and gradual process, *P. glacialis,* as a representative of the polar dinoflagellate, may get used to the new temperature and continue to contribute to the polar dinoflagellate biomass.

4. The growth rate for the culture growing at continuous irradiance was almost equal to the culture growing at normal light/dark cycle. 4 peaks of G_2% and 3 peaks of S% existed in a diel cycle of the CCMP2088 culture growing at 4°C and 24 L, which were 6-8 hours apart from each other. They were more than those growing at normal light/dark cycles. The two indicator proteins expressed circadiumLy. Their expression peaks were at the same time that the first S% peak appeared. The results above indicated that continuous irradiance changed the cell cycle pattern of *P. glacialis*. But the culture could still grow regularly.

In continuous dark the cell densities kept decreasing with 4 smaller peaks of G_2% and S% in a diel cycle. The overall G_2% was lower than the culture growing at 24 L and 14L/10D cycle (P<0.01). In addition, the abundance of Rubisco and PCNA-like proteins per unit persistently declined. Thus complete darkness not only disturbed the cell cycle but also the natural growth. Therefore, *P. glacialis* should annually bloom in the spring of high altitudes with normal light/dark cycles, continue to growing prosperously in summer with polar days, decline in autumn with normal light/dark cycles and form abundant resting spores to survive the lengthy winter with polar nights.

Key words: *Polarella glacialis*; Growth; Cell cycle; Rubisco; PCNA